PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 01 May 2000 (01.05.00)	
International application No. PCT/GB99/02845	**Applicant's or agent's file reference** CAH/4145
International filing date (day/month/year) 27 August 1999 (27.08.99)	**Priority date** (day/month/year) 28 August 1998 (28.08.98)
Applicant BLAKE, David, Russell et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 March 2000 (27.03.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer S. Mafla
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
CAH/4145		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 02845	27/08/1999	28/08/1998
Applicant		
THE UNIVERSITY OF BATH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02845

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 23-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/44 A61K35/20 A23C17/02 A23C11/00 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 645 834 A (COCKRUM RICHARD H) 8 July 1997 (1997-07-08) column 2, line 4 - line 29; claim 17	1-7, 10-14, 18, 20-22,28
X	US 5 310 541 A (MONTGOMERY ROBERT E) 10 May 1994 (1994-05-10) the whole document	1-8, 10-28
X	WO 93 23080 A (FOSSEL ERIC T ;BETH ISRAEL HOSPITAL (US)) 25 November 1993 (1993-11-25) page 4, line 22 -page 6, line 2 page 18, line 11 - line 18	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2000	06/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 518 445 A (GIST BROCADES NV) 16 December 1992 (1992-12-16) page 4, line 9 - line 28 page 6, line 1 - line 3 page 6, line 44 - line 48; claim 17	1-7, 10-14, 18-28
A	EP 0 477 143 A (IDI FARMACEUTICI SPA) 25 March 1992 (1992-03-25) the whole document	1-28
A	MILLAR T.M. ET AL: "Xanthine oxidase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions" FEBS LETTERS,May 1998 (1998-05), pages 225-228, XP002133526 cited in the application the whole document	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/02845

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5645834 A	08-07-1997	CA 2148963 A	10-11-1995
		GB 2289278 A,B	15-11-1995
		IE 950335 A	29-11-1995
US 5310541 A	10-05-1994	AU 4839893 A	29-03-1994
		CA 2143111 A	17-03-1994
		DE 69326955 D	09-12-1999
		EP 0658096 A	21-06-1995
		WO 9405252 A	17-03-1994
WO 9323080 A	25-11-1993	AU 4375293 A	13-12-1993
EP 0518445 A	16-12-1992	AT 136740 T	15-05-1996
		AU 652279 B	18-08-1994
		AU 2275392 A	12-01-1993
		DE 69209894 D	23-05-1996
		DE 69209894 T	05-09-1996
		IE 74397 B	30-07-1997
		JP 6500700 T	27-01-1994
		WO 9222221 A	23-12-1992
		NZ 243106 A	27-09-1994
		US 5747078 A	05-05-1998
EP 0477143 A	25-03-1992	IT 1241994 B	02-02-1994
		AT 136787 T	15-05-1996
		DE 69118796 D	23-05-1996
		DE 69118796 T	24-10-1996
		DK 477143 T	08-07-1996
		ES 2086518 T	01-07-1996

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REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* CAH/4145

Box No. I TITLE OF INVENTION

INGESTIBLE COMPOSITIONS COMPRISING ANTIBACTERIAL AGENTS

Box No. II APPLICANT

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

THE UNIVERSITY OF BATH
CLAVERTON DOWN
BATH
BA2 7AY

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: UNITED KINGDOM

State *(that is, country)* of residence: UNITED KINGDOM

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BLAKE, David Russell
The Ground Floor Flat
16 The Circus
Bath
BA1 2ET

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

Humphreys, Ceris Anne
Abel & Imray
20 Red Lion Street
London
WC1R 4PQ
Further representatives are listed in the Supplemental Box

Telephone No. 0171 242 9984

Facsimile No. 0171 242 9989

Teleprinter No. 24621 Imray G

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

STEVENS, Clifford Robert
49 The Old Batch
Bradford on Avon
Wiltshire
BA15 1TL

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

EISENTHAL, Robert
20 Lansdown Lane
Bath
BA1 4LR

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HARRISON, Roger
16 Grove Leaze
Bradford on Avon
Wiltshire
BA15 1PH

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

MILLAR, Timothy Mark
139 Langdon Road
Southdown
Bath
BA2 1LT

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of:
- ☐ all designated States
- ☐ all designated States except the United States of America
- ☒ the United States of America only
- ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

EDWARDS, Rachel
2 Southville Road
Bradford on Avon
Wiltshire
BA15 1HP

This person is:

- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [X] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:

- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] all designated States except the United States of America [ ] the United States of America only [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information, in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available, in such case, write "Continuation of Box No. III" and indicate, for each additional person, the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**, in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**, in such case, write "Continuation of Box No. IV" and indicate, for each further agent, the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "**patent of addition**," or "**certificate of addition**," or if, in Box No. V, the name of the United States of America is accompanied by an indication "**continuation**" or "**continuation-in-part**", in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**, in such case, write "Continuation of Box No. VI" and indicate, for each additional earlier application, the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**, in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement, in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**, in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

Continuation of Box No. IV - Agent or Common Representative

DARBY	David	Thomas	
COULSON	Anthony	John	
BARRY	Patrick	James	
SENIOR	Janet		
BARDO	Julian	Eason	
MAIR	Richard	Douglas	
LEGG	Cyrus	James	Grahame
CARTER	Caroline	Ann	
NETTLETON	John	Victor	
LOWTHER	Deborah	Jane	
ADAMS	Nicola		
PEARSON	James	Ginn	

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, UG Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakhstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ **AE** United Arab Emirates
☒ **AL** Albania
☒ **AM** Armenia
☒ **AT** Austria
☒ **AU** Australia
☒ **AZ** Azerbaijan
☒ **BA** Bosnia and Herzegovina
☒ **BB** Barbados
☒ **BG** Bulgaria
☒ **BR** Brazil
☒ **BY** Belarus
☒ **CA** Canada
☒ **CH and LI** Switzerland and Liechtenstein
☒ **CN** China
☒ **CU** Cuba
☒ **CZ** Czech Republic
☒ **DE** Germany
☒ **DK** Denmark
☒ **EE** Estonia
☒ **ES** Spain
☒ **FI** Finland
☒ **GB** United Kingdom
☒ **GD** Grenada
☒ **GE** Georgia
☒ **GH** Ghana
☒ **GM** Gambia
☒ **HR** Croatia
☒ **HU** Hungary
☒ **ID** Indonesia
☒ **IL** Israel
☒ **IN** India
☒ **IS** Iceland
☒ **JP** Japan
☒ **KE** Kenya
☒ **KG** Kyrgyzstan
☒ **KP** Democratic People's Republic of Korea
☒ **KR** Republic of Korea
☒ **KZ** Kazakhstan
☒ **LC** Saint Lucia
☒ **LK** Sri Lanka
☒ **LR** Liberia
☒ **LS** Lesotho
☒ **LT** Lithuania
☒ **LU** Luxembourg
☒ **LV** Latvia
☒ **MD** Republic of Moldova
☒ **MG** Madagascar
☒ **MK** The former Yugoslav Republic of Macedonia
☒ **MN** Mongolia
☒ **MW** Malawi
☒ **MX** Mexico
☒ **NO** Norway
☒ **NZ** New Zealand
☒ **PL** Poland
☒ **PT** Portugal
☒ **RO** Romania
☒ **RU** Russian Federation
☒ **SD** Sudan
☒ **SE** Sweden
☒ **SG** Singapore
☒ **SI** Slovenia
☒ **SK** Slovakia
☒ **SL** Sierra Leone
☒ **TJ** Tajikistan
☒ **TM** Turkmenistan
☒ **TR** Turkey
☒ **TT** Trinidad and Tobago
☒ **UA** Ukraine
☒ **UG** Uganda
☒ **US** United States of America
☒ **UZ** Uzbekistan
☒ **VN** Viet Nam
☒ **YU** Yugoslavia
☒ **ZA** South Africa
☒ **ZW** Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ Costa Rica
☒ Dominica

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 28/8/98 (28 August 1998)	9818913.7	United Kingdom (GB)		
item (2) 10/12/98 (10 Dec. 1998)	9827243.8	United Kingdom (GB)		
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s). (1) and (2)

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*: ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority)*.

Date *(day month year)* | Number | Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	6
description (excluding sequence listing part)	33
claims	3
abstract	1
drawings	4
sequence listing part of description	
Total number of sheets	47

This international application is **accompanied** by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify)*:

Figure of the drawings which should accompany the abstract: 3

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Ceris Anne Humphreys

N Adams

NICOLA ADAMS (AGENT)

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	
4. Date of timely receipt of the required corrections under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

...mand must be filed directly with the c... ent International Preliminary Examining Authority... two or more Authorities are competent, ...with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below.

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference
		CAH/4145WO
International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/GB99/02845	27/08/1999 (27 August 1999)	28/08/1998(28 August 1998)

Title of invention
INGESTIBLE COMPOSITIONS COMPRISING ANTIBACTERIAL AGENTS

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

THE UNIVERSITY OF BATH
Claverton Down
Bath
BA2 7AY
UNITED KINGDOM

Telephone No.:

Facsimile No.:

Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BLAKE, David Russell
The Ground Floor Flat
16 The Circus
Bath
BA1 2ET
UNITED KINGDOM

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

STEVENS, Clifford Robert
49 The Old Batch
Bradford on Avon
Wiltshire
BA15 1TL
UNITED KINGDOM

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
GB	GB

[X] Further applicants are indicated on a continuation sheet.

See Notes to the demand form

Sheet No 2

International application No
PCT/GB99/02845

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

EISENTHAL, Robert
20 Lansdown Lane
Bath
BA1 4LR
United Kingdom

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HARRISON, Roger
16 Grove Leaze
Bradford on Avon
Wiltshire
BA15 1PH
United Kingdom

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MILLAR, Timothy Mark
139 Langdon Road
Southdown
Bath
BA2 1LT
United Kingdom

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

EDWARDS, Rachel
2 Southville Road
Bradford on Avon
Wiltshire
BA15 1HP
United Kingdom

State *(that is, country)* of nationality: GB

State *(that is, country)* of residence: GB

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/GB99/02845

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier

Name and address *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

HUMPHREYS, Ceris Anne
Abel & Imray
20 Red Lion Street
London WC1R 4PQ

United Kingdom

Telephone No
0207 242 9984

Facsimile No
0207 242 9989

Teleprinter No.
24621 IMRAY G

[ ] **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special addr ess to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination to start on the basis of:

[X] the international application as originally filed

the description
[ ] as originally filed
[ ] as amended under Article 34

the claims
[ ] as originally filed
[ ] as amended under Article 19 (together with any accompanying statement)
[ ] as amended under Article 34

the drawings
[ ] as originally filed
[ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [ ] The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: English

[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects all eligible States *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes not to elect:

See Notes to the demand form

International application No
PCT/GB99/02845

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination.			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	sheets	☐	☐
2. amendments under Article 34	:	sheets	☐	☐
3. copy (or, where required, translation) of amendments under Article 19	:	sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	sheets	☐	☐
5. letter	:	sheets	☐	☐
6. other *(specify)*	:	sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Ceris Anne Humphreys

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
ABEL & IMRAY
Attn. HUMPHREYS, C.
20 Red Lion Street
London WC1R 4PQ
UNITED KINGDOM

4145
JJC

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference CAH/4145	Date of mailing *(day/month/year)* 06/04/2000
International application No. PCT/GB 99/02845	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant THE UNIVERSITY OF BATH et al.	International filing date *(day/month/year)* 27/08/1999

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41–22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL–2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Nina Vercio

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
CAH/4145		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 02845	27/08/1999	28/08/1998
Applicant		
THE UNIVERSITY OF BATH et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02845

Box I Observations where certain claims w re found unsearchabl (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 23-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees w re accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/GB 99/02845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/44 A61K35/20 A23C17/02 A23C11/00 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 645 834 A (COCKRUM RICHARD H) 8 July 1997 (1997-07-08) column 2, line 4 - line 29; claim 17	1-7, 10-14, 18, 20-22,28
X	US 5 310 541 A (MONTGOMERY ROBERT E) 10 May 1994 (1994-05-10) the whole document	1-8, 10-28
X	WO 93 23080 A (FOSSEL ERIC T ;BETH ISRAEL HOSPITAL (US)) 25 November 1993 (1993-11-25) page 4, line 22 -page 6, line 2 page 18, line 11 - line 18	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2000	06/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 518 445 A (GIST BROCADES NV) 16 December 1992 (1992-12-16) page 4, line 9 - line 28 page 6, line 1 - line 3 page 6, line 44 - line 48; claim 17	1-7, 10-14, 18-28
A	EP 0 477 143 A (IDI FARMACEUTICI SPA) 25 March 1992 (1992-03-25) the whole document	1-28
A	MILLAR T.M. ET AL: "Xanthine oxidase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions" FEBS LETTERS,May 1998 (1998-05), pages 225-228, XP002133526 cited in the application the whole document	1-28

INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No PCT/GB 99/02845

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5645834 A	08-07-1997	CA 2148963 A	10-11-1995
		GB 2289278 A,B	15-11-1995
		IE 950335 A	29-11-1995
US 5310541 A	10-05-1994	AU 4839893 A	29-03-1994
		CA 2143111 A	17-03-1994
		DE 69326955 D	09-12-1999
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04582

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 45/05, 39/00, 37/48, 37/62; C12N 9/02
US CL :424/94.2, 94.1, 94.3, 85.1, 85.2, 85.91; 435/189

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.2, 94.1, 94.3, 85.1, 85.2, 85.91; 435/189; 514/410, 185

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, Medline, Biosis, Registry

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,906,469 (Jansen, et al.) 06 March 1990, see entire document, especially col. 1, lines 26-31, col. 5, lines 11-12, col.6, lines 6-8 and 54-64, and col. 13, lines 6-9.	1-23
Y	MOLECULAR AND CELLULAR BIOCHEMISTRY, Vol. 10(1), issued 31 January 1976, A. Bozzi, et al., "Enzyme Defense Against Reactive Oxygen Derivatives. II. Erythrocytes and Tumor Cells," pages 11-16, especially pages 11 and 12.	1-23
Y	US, A, 4,971,991 (Umemura, et al.) 20 November 1990, see entire document.	1-23
Y	US, A, 4,975,278 (Senter, et al.) 04 December 1990, see entire document.	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

21 JULY 1993

Date of mailing of the international search report

29 JUL 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

KRISTIN K. LARSON

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,762,707 (Jansen, et al.) 09 August 1988, see entire document.	1-23
A	US, A, 4,937,183 (Ultee, et al.) 26 June 1990.	1-23
A	US, A, 4,671,958 (Rodwell, et al.) 09 June 1987.	1-23
A	US, A, 4,867,973 (Goers, et al.) 19 September 1989.	1-23
A	ACCOUNTS OF CHEMICAL RESEARCH, Vol. 5(10), issued October 1972, I. Fridovich, "Superoxide Radical and Superoxide Dismutase," pages 321-326.	1-23

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

HUMPHREYS, C.
ABEL & IMRAY
20 Red Lion Street
London WC1R 4PQ
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	28.12.2000
Applicant's or agent's file reference CAH/4145	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02845	27/08/1999	28/08/1998

Applicant
THE UNIVERSITY OF BATH et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Tantum, P Tel.+49 89 2399-8143

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CAH/4145		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/02845	27/08/1999	28/08/1998
International Patent Classification (IPC) or national classification and IPC A23L1/00		
Applicant THE UNIVERSITY OF BATH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/03/2000	28.12.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Smeets, D Telephone No. +49 89 2399 7492

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/02845

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-33 as originally filed

Claims, No.:

1-35 as received on 04/12/2000 with letter of 29/11/2000

Drawings, sheets:

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 27-31 with regard to industrial applicability.

because:

☒ the said international application, or the said claims Nos. 27-31 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 2, 5, 12-14, 27-29

	No:	Claims	1, 3, 4, 6-11, 15-26, 30-35
Inventive step (IS)	Yes:	Claims	28
	No:	Claims	1-27, 29-35
Industrial applicability (IA)	Yes:	Claims	1-26, 32-35
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
s e separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 27-31 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: US-A-5 645 834 (COCKRUM RICHARD H) 8 July 1997 (1997-07-08)
D2: US-A-5 310 541 (MONTGOMERY ROBERT E) 10 May 1994 (1994-05-10)
D3: MILLAR T.M. ET AL: 'Xanthine oxidase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions' FEBS LETTERS,May 1998 (1998-05), pages 225-228, XP002133526 cited in the application
D4: EP-A-0 518 445 (GIST BROCADES NV) 16 December 1992 (1992-12-16)
D5: Souci, Fachmann, Kraut : Food Composition and Nutrition Tables, p.14,15,45,46. Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1989

1) Remarks concerning claims 1-18, 26-31, 33-35 with regard to industrial applicability

For the assessment of the present claims 1-18, 26-31, 33-35 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2) Novelty - Art. 33(1) and (2) PCT

The subject-matter of amended claims 1, 3, 4, 6-11, 15-26, 30-35 does not fulfill the requirements of Article 33(2) PCT.

The subject-matter of claims 1, 6-11, 16-26, 30-35 is not new since pasteurized bovine milk, milk powder, lyophilized butter milk, colostrum, buttermilk and human milk comprise active xanthine oxidoreductase, electron acceptors and donors (e.g. even H_2O can act as an electron acceptor or an electron donor). All kinds of milk and milk powders contain electron donors and electron acceptors such organic acids, amino acids, non-protein nitrogenous constituents (ammonia, nitrates), minerals. See for instance, D5, page 14, 15, 45, 46. This is also acknowledged in the description of the present application (page 33, lines 15-16). It is common knowledge that pasteurized milk is administered to infants and adults. These natural products (pasteurized bovine milk, milk powder, colostrum, butter milk, lyophilized buttermilk, spray-dried buttermilk and human milk) are known in the art and they are suitable for a feed formulation, formula feed or enteral feed.
The mixing of lyophilized or spray-dried milk, buttermilk with water is generally known in the art. Spray-drying techniques that avoid denaturation of proteins and inactivation of xanthine oxidase are also generally known in the art.
It could be argued that cow's milk is not suitable for consumption by young babies, but a formula feed is not restricted to be administered only to young babies. A two-year-old infant can be fed with milk and with formula feed, especially manufactured for children at this age. In addition, it is generally known that diluted cow's milk and mother's milk are suitable for administration to young babies. Consequently, these compositions anticipate the subject-matter of independent claim 1, even if a formula feed is regarded as suitable only for young babies. The description of the present application (page 3, line 24-25, 30-34, page 4, line 1) mentions that babies can be fed with formula feed, and that a formula feed also comprises compositions that are not nutritionally complete.

In addition, the subject-matter of claims 1, 3, 4, 7-9, 11, 15, 16, 18-21, 24-26, 33-35 lacks novelty in view of D1.
D1 (column 2, lines 10, 35-37, column 17, lines 32-43, column 18, lines 59-66) discloses a dietary supplement for calves, comprising proteins derived from colostrum. Xanthine oxidase is disclosed as a protein present in colostrum and it is also disclosed

that said xanthine oxidase has antimicrobial activity, particularly in the gut. It is implicit that the enzyme has not been inactivated since the method of preparation does not disclose a process (e.g. heat treatment) that could inactivate the xanthine oxidoreductase (column 3, lines 25-58).
The colostrum used in D1 is defined to be the secretion of the mammary glands which is produced during the first few days of lactation. Since this supplement is administered to calves, the supplement is considered to be suitable for a formula feed and enteral feed. Furthermore, the composition implicitly comprises components that can as electron acceptors and/or electron donors.
The subject-matter of claim 3 lacks novelty in view of D1, since said document (column 18, lines 59-66, column 3, lines 18-21, 44-47) discloses a purified protein-rich whey product, derived from colostrum and comprising xanthine oxidase (see list column 18, lines 65-67). It is implicit that in said concentrated product, the concentration of xanthine oxidoreductase (XOR) exceeds the normal physiological concentration of XOR.
In D1, the antimicrobial activity of xanthine oxidoreductase in the gut is emphasized. Therefore, said composition is also suitable for use in the treatment of Scours disease and gastrointestinal infection. D1 further (column 3, lines 51-55) discloses that the resulting product, derived from colostrum and comprising xanthine oxidase, is whey, which is a liquid.
The filter sterilized concentrated whey product, comprising XOR, is stored in a refrigerating tank (column 3, lines 42-45). A physiological saline solution is added to dilute the protein content to 6-7% before administration (column 3, lines 48-51). It is implicit that this saline solution is also sterile since it is being added to a sterilized composition. This means also that these two portions are held separate until use.

D2 (abstract, column 3, lines 1-26, 34) discloses an antimicrobial animal chew, comprising an oxidoreductase and its corresponding substrate. Xanthine oxidase is disclosed as a suitable oxidoreductase (see list column 3, 30-35). Said document also discloses that the solid, chewable carrier is preferably durable enough to prevent the animal from consuming it in less than about 1 to 5 minutes (column 3, lines 24-26). D2 discloses a coating comprising xanthine oxidase (see list column 7, lines 9-16) which is coated on a chewable carrier. This composition is suitable for use as a bactericidal agent and in the treatment of Scours disease.
Therefore, the subject-matter of claims 33 and 35 is also not new in view of D2.

The subject-matter of claim 32 is not new in the light of D1 and D2, since both documents (D1, column 17, lines 32-43 and D2, column 3, lines 4-8, 34) disclose a formulation comprising XOR (see also **Re Item VIII**).

D4 (page 4, line 27; page 6, lines 1, 48) discloses xanthine oxidase and its corresponding substrate and its use in feedstuff.

The subject-matter of claims 2, 5, 12-14, 27-29 is new in view of the documents of the search report.

3) Inventive Step - Art. 33(1) and (3) PCT

The subject-matter of claims 1-27, 29-35 does not fulfill the requirements of Article 33(3) PCT.

The technical problem of this invention is providing an alternative formulation comprising active xanthine oxidoreductase (XOR) for use as a human or animal feed.
D1 is being regarded as closest prior art.
The additional technical feature of claims 2 and 5 is a formulation having a particular XOR concentration.
D1 (column 2, lines 10-12, column 3, lines 11-13, 25-55) provides a solution by providing a whey product, comprising xanthine oxidase for administration to calves. D1 also discloses a dried diet supplement, derived from colostrum protein whey.
Since it cannot be seen which technical problem is solved by having a particular XOR concentration, there is no evidence for the presence of an inventive step in claim 2 and 5.

D1 emphasizes the antimicrobial activity of xanthine oxidase in the gut and states that a whey product comprising this enzyme has beneficial effects, even in adult cows.
Therefore, it is obvious to the skilled man to use xanthine oxidase for the treatment of gastrointestinal infection.
Consequently, the subject-matter of claims 27 and 29 does not involve an inventive step.
The subject-matter of claims 12-14 is trivial to the skilled man
The feature "powder" in claim 12 is merely one of several straightforward possibilities

from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.
The features "heat treated" or "pasteurized" in claims 13 and 14 are merely straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed.

In addition, D2 (column 3, lines 1-30, column 4, lines 53-64) mentions that the xanthine oxidase composition may also include thiocyanate ions. In that case, an oxidoreductase system is prepared, comprising electron donors and acceptors (see mechanism D2, column 2, lines 45-61).
Therefore, the combination of electron donors and electron acceptors with xanthine oxidoreductase is regarded as obvious.

Since the use of XOR in the treatment of Scours disease (enteric diseases of swines) is not disclosed in the documents of the search report, the subject-matter of claim 31 is considered to be inventive.

Re Item VII
Certain defects in the international application

1) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1, D2 and D4 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

Claim 32 contains a reference to the description. According to Rule 6.2(a) PCT, claims should not contain such references except where absolutely necessary, which is not the case here.
Therefore, the subject-matter of claim 34 is regarded as having only one technical feature, namely a formulation comprising xanthine oxidoreductase.

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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9827243.8	10 December 1998 (10.12.98)	GB

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF BATH [GB/GB]; Claverton Down, Bath BA2 7AY (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BLAKE, David, Russell [GB/GB]; The Ground Floor Flat, 16 The Circus, Bath BA1 2ET (GB). STEVENS, Clifford, Robert [GB/GB]; 49 The Old Batch, Bradford on Avon, Wiltshire BA15 1TL (GB). EISENTHAL, Robert [GB/GB]; 20 Lansdown Lane, Bath BA1 4LR (GB). HARRISON, Roger [GB/GB]; 16 Grove Leaze, Bradford on Avon, Wiltshire BA15 1PH (GB). MILLAR, Timothy, Mark [GB/GB]; 139 Langdon Road, Southdown, Bath BA2 1LT (GB). EDWARDS, Rachel [GB/GB]; 2 Southville Road, Bradford on Avon, Wiltshire BA15 1HP (GB).

(74) Agents: HUMPHREYS, Ceris, Anne et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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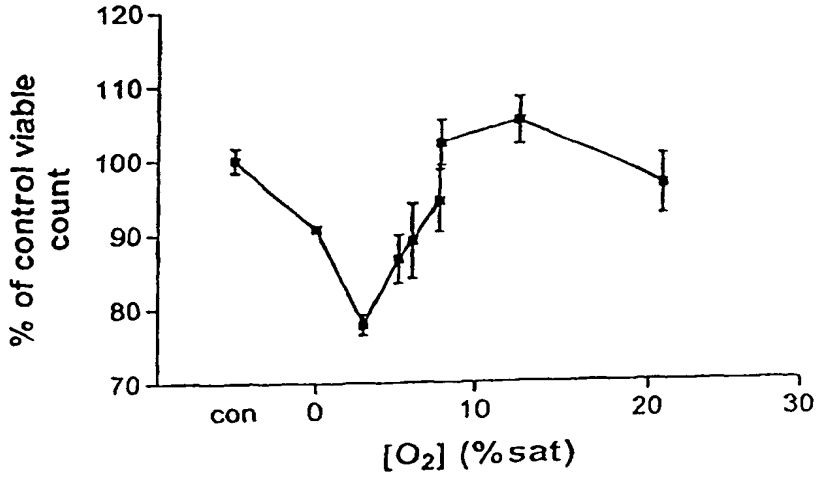
Without international search report and to be republished upon receipt of that report.

(54) Title: INGESTIBLE COMPOSITIONS COMPRISING ANTIBACTERIAL AGENTS



(57) Abstract

A formulation for use as a bactericidal agent in the human or animal digestive system includes xanthine oxidoreductase. The formulation may especially be in the form of a formula feed formulation or enteral feed formulation for administration to a human or animal. The formulation is capable of functioning as a "natural antibiotic" to prevent or reduce bacterial infection within the gut, especially the neonatal gut.

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US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

Claims:

1. A formulation for use as a bactericidal agent in the human or animal digestive system, the formulation including active xanthine oxidoreductase (XOR).

2. A formula feed formulation or enteral feed formulation for administration to a human or animal, the formulation including active xanthine oxidoreductase (XOR).

3. A formulation according to claim 2, in which the formulation is for use as formula feed.

4. A formulation according to any one of claims 1 to 3, in which the concentration of XOR exceeds the normal physiological concentration of XOR.

5. A formulation according to any one of claims 1 to 4, in which the formulation includes from 50 to 150μg/ml of XOR.

6. A formulation according to any one of claims 1 to 5, in which the formulation includes buttermilk, the buttermilk including active XOR.

7. A formulation according to any one of claims 1 to 6, in which the formulation is in liquid form.

8. A formulation according to any one of claims 1 to 7, the formulation further including one or more electron donors.

9. A formulation according to any one of claims 1 to 8, the formulation further including one or more electron acceptors.

10. A combination product for use in the preparation of a formulation according to any one of claims 1 to 9, in which the product comprises two separate portions, the first portion including active XOR and the second portion comprising substantially no active XOR.

11. A combination product according to claim 10, in which the second portion is in the form of a powder.

12. A formulation according to claim 10 or claim 11, in which the second portion has been heat treated.

13. A formulation according to any one of claims 10 to 12, in which the first portion has been pasteurised.

14. A formulation according to any one of claims 10 to 13, in which the first portion is in a first container, the second portion is in a second container.

15. A composition for addition to a formulation for use as feed, the composition comprising active XOR in combination with one or more electron donors and/or one or more electron acceptors.

16. A composition according to claim 15, the composition comprising buttermilk.

17. A composition according to claim 15 or claim 16, the composition being in the form of a powder.

18. A method of making a formulation for use as feed, the method comprising the step of adding a composition comprising active XOR.

19. A method according to claim 18, the composition being according to any one of claims 15 to 17.

20. A method according to claim 19 or claim 20, the method comprising the steps of:

a. preparing a first portion of the formulation, the first portion comprising a composition including active XOR; and

b. preparing a second portion of the formulation, the first portion and second portion being separate from each other for subsequent mixing to form the formulation.

21. A method according to claim 20, in which the first portion comprises lyophilised buttermilk.

22. A method according to claim 20 or claim 21, in which the second portion comprises a treated feed composition.

23. Use of active XOR in the treatment of gastrointestinal (GI) infection.

24. Use of active XOR in the treatment of Scours disease.

25. Use of active XOR in the killing of bacteria.

26. Method of feeding a patient with enteral feed, in which the enteral feed includes active XOR.

27. Method of feeding an infant with formula feed, in which the formula feed includes active XOR.

28. A formulation comprising XOR substantially as described in Example 1 herein.

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(71) Applicant *(for all designated States except US)*: **THE UNIVERSITY OF BATH** [GB/GB]; Claverton Down, Bath BA2 7AY (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BLAKE, David, Russell** [GB/GB]; The Ground Floor Flat, 16 The Circus, Bath BA1 2ET (GB). **STEVENS, Clifford, Robert** [GB/GB]; 49 The Old Batch, Bradford on Avon, Wiltshire BA15 1TL (GB). **EISENTHAL, Robert** [GB/GB]; 20 Lansdown Lane, Bath BA1 4LR (GB). **HARRISON, Roger** [GB/GB]; 16 Grove Leaze, Bradford on Avon, Wiltshire BA15 1PH (GB). **MILLAR, Timothy, Mark** [GB/GB]; 139 Langdon Road, Southdown, Bath BA2 1LT (GB). **EDWARDS, Rachel** [GB/GB]; 2 Southville Road, Bradford on Avon, Wiltshire BA15 1HP (GB).

(74) Agents: **HUMPHREYS, Ceris, Anne** et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

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(54) Title: INGESTIBLE COMPOSITIONS COMPRISING ANTIBACTERIAL AGENTS

(57) Abstract: A formulation for use as a bactericidal agent in the human or animal digestive system includes xanthine oxidoreductase. The formulation may especially be in the form of a formula feed formulation or enteral feed formulation for administration to a human or animal. The formulation is capable of functioning as a "natural antibiotic" to prevent or reduce bacterial infection within the gut, especially the neonatal gut.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/GB 99/02845

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/44 A61K35/20 A23C17/02 A23C11/00 A61P31/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 645 834 A (COCKRUM RICHARD H) 8 July 1997 (1997-07-08) column 2, line 4 - line 29; claim 17	1-7, 10-14, 18, 20-22,28
X	US 5 310 541 A (MONTGOMERY ROBERT E) 10 May 1994 (1994-05-10) the whole document	1-8, 10-28
X	WO 93 23080 A (FOSSEL ERIC T ;BETH ISRAEL HOSPITAL (US)) 25 November 1993 (1993-11-25) page 4, line 22 -page 6, line 2 page 18, line 11 - line 18	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2000	06/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fernandez y Branas,F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 99/02845

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 518 445 A (GIST BROCADES NV) 16 December 1992 (1992-12-16) page 4, line 9 - line 28 page 6, line 1 - line 3 page 6, line 44 - line 48; claim 17	1-7, 10-14, 18-28
A	EP 0 477 143 A (IDI FARMACEUTICI SPA) 25 March 1992 (1992-03-25) the whole document	1-28
A	MILLAR T.M. ET AL: "Xanthine oxidase catalyses the reduction of nitrates and nitrite to nitric oxide under hypoxic conditions" FEBS LETTERS,May 1998 (1998-05), pages 225-228, XP002133526 cited in the application the whole document	1-28

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/02845

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 23-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. onal Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE 69118796 T	24-10-1996
		DK 477143 T	08-07-1996
		ES 2086518 T	01-07-1996

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF BATH [GB/GB]; Claverton Down, Bath BA2 7AY (GB).

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(74) Agents: HUMPHREYS, Ceris, Anne et al.; Abel & Imray, 20 Red Lion Street, London WC1R 4PQ (GB).

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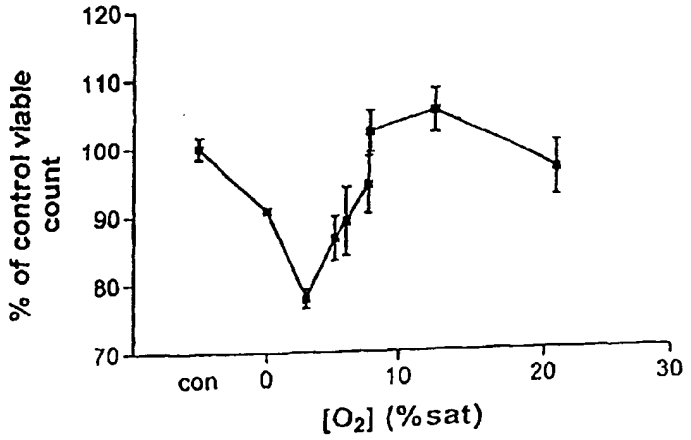

(57) Abstract

A formulation for use as a bactericidal agent in the human or animal digestive system includes xanthine oxidoreductase. The formulation may especially be in the form of a formula feed formulation or enteral feed formulation for administration to a human or animal. The formulation is capable of functioning as a "natural antibiotic" to prevent or reduce bacterial infection within the gut, especially the neonatal gut.

Ingestible compositions comprising antibacterial agents

The present invention relates to compositions comprising xanthine oxidoreductase (XOR). In particular, but not exclusively, the invention relates to the use of a composition comprising XOR as a feed for humans or animals, and finds special application in relation to formula feed for babies.

The enzyme xanthine oxidoreductase (XOR) is a complex molybdoflavoprotein, the action of which has been studied for many years.

XOR is a major protein component of the membrane surrounding fat droplets in whole milk. Consequently, cows' milk is a rich and convenient source of the enzyme, as has been known for many years. XOR has also been characterised from rat, chicken and turkey livers. More recently, human milk has been found to contain XOR, which has now been purified from that source. Initial research shows that, while the human milk XOR enzyme has similar physicochemical properties to the bovine milk enzyme, the human enzyme shows differences in its catalytic activity.

The purpose of XOR in milk has never been fully ascertained. One suggestion has been that it provides a source of molybdenum and iron, metals potentially useful for the developing neonate.

There have also been suggestions that XOR has a role in the production of bactericidal agents. As discussed further below, XOR can catalyse the production of superoxide and hydrogen peroxide, which are known bactericidal agents. As indicated below, however, in the low oxygen concentrations found in the gut, it is thought

unlikely that bactericidal levels of superoxide or hydrogen peroxide will be attained.

The role of XOR in the development of gout has been the subject of extensive study. XOR is involved in the catabolism of purines to uric acid, catalysing the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Compositions have been developed which block the action of XOR and which are useful in the prevention and treatment of gout.

XOR exists in two inter-convertible forms, xanthine dehydrogenase (XDH, EC 1.1.1.204) and xanthine oxidase (XO, EC 1.1.3.22). XDH, which is believed to predominate *in vivo*, preferentially reduces NAD^+, whereas XO does not reduce NAD^+, preferring molecular oxygen. Where reference is made herein to xanthine oxidoreductase, it should be understood that that term refers to both xanthine dehydrogenase (XDH) and xanthine oxidase (XO), where appropriate. It will be appreciated that references to XOR further include references to analogs of xanthine oxidoreductase that have xanthine oxidoreductase activity. Such analogs may include but are not limited to, for example, xanthine oxidoreductase which has been modified chemically or otherwise, analogs having fragments of xanthine oxidoreductase derived from naturally-occurring enzyme, and analogs having polypeptides obtained by replication of the enzyme or a portion thereof using any suitable biotechnological method, provided in each case that the catalytic activity of the endogenous xanthine oxidoreductase is retained at least to an appreciable extent.

XOR can also effect reduction of molecular oxygen. That reaction generates the reactive oxygen species superoxide anion and hydrogen peroxide.

The superoxide radicals thus generated have been implicated in relation to chronic inflammatory intestinal diseases such as Crohn's disease and colitis ulcerosa. US 5,484,605 describes the administration of oxypurinol to the intestine to inhibit xanthine oxidase, thus preventing the formation of superoxide radicals which are believed to act as inflammatory mediators.

It has been reported, Millar, T.M. *et al*, FEBS Letters 427 (1998) 225-228, that, under hypoxic conditions and in the presence of NADH, XOR is capable of catalysing the reduction of glyceryl trinitrate (GTN), as well as inorganic nitrate and nitrite, to nitric oxide (NO).

Nitric oxide (NO) is widely recognised as mediating the relaxation of smooth muscle in vasodilation and as initiating many other important biological functions, including inhibition of platelet aggregation and adhesion. Its generally accepted physiological source is NO synthase, a complex enzyme which is totally dependent on oxygen for its activity and consequently ineffective in a hypoxic environment, where the vasodilatory properties of NO might be seen to be advantageous.

Babies who are not breast-fed are fed what is referred to herein as formula feed. Such formula feed has a composition which commonly includes sources of protein, fat and carbohydrate as well as minerals and are generally formulated to be nutritionally complete. Many formula feeds are based on cow's milk while some others are soy based. The term "formula feed" used herein should be understood to cover both formulations based on milk products as well as those based on soya or other products and which may or may not be nutritionally

complete. The formula feed commonly takes the form of a dried powder which is reconstituted before being fed to the baby. Alternatively, the formula feed may be in liquid form either as a concentrated liquid requiring dilution or as a ready-to-feed formulation.

Enteral feeding may be prescribed where a patient is unable to eat normally, or has severe malabsorption or malnourishment. Enteral feed may take the form of tube and sip feeds. The enteral feed may be nutritionally complete and generally comprises protein, carbohydrate and fat as well as vitamins and minerals. The term "enteral feed" used herein should be understood to cover both formulations based on milk products as well as those based on soya or other products and which may or may not be nutritionally complete. The enteral feed may be in liquid form or may be in the form of a powder which is reconstituted before use. In many cases the enteral feed formulation is based on the composition of the neonatal formula feed.

The present invention provides a formulation for use as a bactericidal agent in the human or animal digestive system, the formulation including active xanthine oxidoreductase.

More particularly, in accordance with the invention, there is provided a formula feed formulation or enteral feed formulation for administration to a human or animal, the formulation including active xanthine oxidoreductase (XOR).

The term "feed" is used herein to include both enteral feeds and formula feeds.

We have found that the active enzyme xanthine oxidoreductase (XOR) is absent from formula feeds and from enteral feeds. It is thought that that is because

XOR is either absent from the feed or it is inactivated as a result of the treatment process used in the production of feeds. Where reference is made herein to "active" XOR and "active" enzyme, it is to be understood that reference is made to XOR and enzyme which has not, for example, been inactivated or broken down in such a way.

Up to now, it would have been thought that the addition of active XOR to feeds would be unnecessary and undesirable. The only roles of XOR previously known, as indicated above, were as a general "housekeeping" enzyme in the catabolism of purines, which may also have a pathological role in the development of gout and of chronic inflammatory intestinal diseases. The only other beneficial role of XOR was thought to be as a source of molybdenum and iron. It was thought (correctly) that those minerals could still be obtained from inactivated XOR, which might be present in feed.

We have now found that the addition of active XOR to feed is potentially beneficial in killing pathogenic intestinal bacteria. The active XOR may thus be regarded as a "natural antibiotic", that is, a substance of natural origin which is capable of destroying or inhibiting the growth of at least some strains of pathogenic micro-organism. It is believed that feed containing active XOR will be beneficial in the mitigation of intestinal infection and necrotising enterocolitis in the neonate fed with formula feed. Sick adults that are enterally fed are also at risk of the same complications as children fed formula feed.

Studies have shown that babies who are fed with formula feed are about twenty times more likely to suffer from gastrointestinal (GI) infection than babies who are

breast fed. The reason for that discrepancy has not been known.

In the presence of oxygen, as stated above, XOR can generate superoxide and hydrogen peroxide. However, the amount of oxygen available in the gut is generally very low and bactericidal levels of superoxides or hydrogen peroxide are unlikely to be attained.

As discussed above, it has been found that the catalysis by XOR of the reduction of glyceryl trinitrate (GTN), as well as inorganic nitrate and nitrite, to nitric oxide (NO) occurs under hypoxic conditions. It is such hypoxic conditions which are present in the gut.

We have also found that the optimum pH for the production of NO under the hypoxic conditions is about pH 5.5. It is known that the pH of the neonatal stomach normally ranges between pH 3.5 and 6. Thus, we have found that the neonatal stomach presents an environment in which the production of NO in the presence of XOR is at a peak. Furthermore, it is at pH levels above about 4 at which pathogenic bacteria are more active than at lower pH of about 2 normally found in the adult stomach. Thus it is believed that the neonatal stomach, having a relatively high pH, is at particular risk from pathogenic bacteria. Furthermore, it is found that, for example in post-operative adults, the pH of the stomach rises to above 4. It has also been noted that post-operative adults have a relatively high risk of GI infection.

Thus, surprisingly, we have found under the conditions of the neonatal gut, XOR can catalyse the production not only of superoxide, but of NO. Superoxide and NO rapidly interact to generate peroxynitrite, a much more potent bactericidal species than superoxide, NO or hydrogen peroxide. While superoxide has some

bactericidal properties and in some situations NO has also been found to kill or damage bacteria, it is the interaction of superoxide and NO to form peroxynitrite and other products which is believed to give superior bactericidal action. Peroxynitrite (and, it is thought, other products of the interaction of superoxide and NO) are particularly potent bactericidal species.

The concentration of nitrite present in the neonatal gut is normally low, and it might therefore be thought that the potential for XOR-catalysed generation of NO would be limited. It is believed however that, the known affinity of XOR for acidic polysaccharides such as those occurring in bacterial capsules causes XOR to become more concentrated in the immediate vicinity of bacteria. In anaerobic environments, bacteria commonly are found to excrete nitrite and thus the association of XOR with the bacteria may have the result that the XOR will be located in a localised region of elevated nitrite concentration.

In one embodiment of the invention, the formulation is for use as formula feed. As indicated above, the use of XOR finds particular application in relation to the feeding of neonates.

Alternatively, the formulation is for use as an enteral feed for adults.

While reference is made herein to the feeding of humans, the invention is also of particular relevance in the feeding of animals and the terms "formula feed", "enteral feed" and "feed" should be understood to include formulations for animals. The invention finds particular application in respect of mammals.

Animals also suffer from GI infection, in particular Scours disease (diarrhoea in neonatal animals). Scours disease is a particular problem for calves and pigs taken

from their mothers soon after birth. For calves and pigs taken from their mothers up to ten days after birth and fed waste milk or formula feed, the mortality rate can be as much as 80%. In some cases, Scours disease can be cured by administering electrolyte solutions but such treatment is very expensive.

It is thought that the addition of active XOR to feed for animals will mitigate GI infections, in particular Scours disease.

The formulation may advantageously include an amount of active XOR which is such that the active XOR concentration in the formulation is at least as large as the normal physiological concentration of XOR, for example in milk. Preferably, the concentration of the XOR exceeds the normal physiological concentration of XOR. Advantageously, the formulation includes from 50 to 500μg/ml of XOR, based on the volume of the formulation (when ready-to-use, having been diluted if necessary). Preferably the formulation includes from 50 to 150μg/ml which is comparable to the level of XOR found in natural breast milk. In some cases, it may be desirable to increase or reduce the amount of active XOR in the feed, for example, as a function of the baby's age and/or the incidence of GI infection.

We have found that a particularly advantageous source of active XOR is buttermilk, in particular lyophilised buttermilk.

In some cases it is thought that the addition of XOR in purified or other form will be desirable, for example where there is a risk of allergy to buttermilk.

The formula feed and/or the enteral feed including the active XOR in accordance with the invention may be in

the form of a powder or may be in liquid form ready for feeding to the patient.

As indicated above, it is believed that for the formula feed and enteral feed formulations, any active XOR which was naturally present in those formulations is destroyed or deactivated during the manufacture of the formulation, possibly as a result of heat treatment. In a particularly preferred embodiment of the invention, one portion of the formula or enteral feed is prepared in the standard way, for example including heat treatment step(s). A composition comprising active XOR is subsequently added to the prepared portion. That addition of the XOR may be carried out by a manufacturer, or may be carried out, for example, immediately prior to use of the formulation. For example, in the case of a powdered feed which is made up with water prior to use, a powdered composition comprising the active XOR may be contained in a separate container from that of the rest of the formulation, for example in a sachet. Alternatively, the portion including the active XOR may be in tablet form or may be contained in a capsule. The rest of the feed may be made up in the normal way, for example by the addition of hot water and shaking, and the XOR composition added once the rest of the feed has been prepared. That is of particular importance in the case in which the normal method of making up of the formulation might deactivate the XOR, for example as a result of the addition of boiling water. Where the making up of the formulation would not damage the activity of the XOR, or where the formulation is sold ready-prepared, the XOR composition and the rest of the formulation may be provided together as a mixture.

Thus the invention provides a combination product for use in the preparation of a formulation in which the product comprises two separate portions, the first portion including active XOR and the second portion comprising substantially no active XOR.

In one embodiment of the invention, the second portion of the formulation is in the form of a powder.

As indicated above, the second portion may have been treated by heat treatment, for example UHT treatment, in the normal way, thus deactivating the XOR.

In a particularly preferred embodiment of the invention, the active XOR is added in the form of buttermilk. The first portion may be pasteurised. As indicated above, pasteurisation has been found not to deactivate XOR.

As indicated above, advantageously, the first portion is in a first container, the second portion is in a second container. Thus the two portions can be held separate until use. For example, where both portions are in the form of a powder, each powder portion can be made up separately using different methods before being mixed together. For example, the first portion might be made up with cold water and the second portion may be made up with boiling water. Alternatively, the first portion may be in the form of a liquid ready for mixing into the second portion of the formulation once the second portion has been made up.

Advantageously, the formulation further includes electron donors and/or electron acceptors. Examples of electron donors are purines and nitrogen-containing heterocyclic compounds, for example hypoxanthine, NADH. Examples of electron acceptors are organic and inorganic nitrates and nitrites.

According to the invention, there is also provided a composition for addition to a formulation for use as feed, the composition comprising active XOR. It is envisaged that the composition containing the active XOR might be sold separately from the formula or enteral feed, the composition being for addition to the feed prior to use.

Advantageously, the composition comprises buttermilk. As indicated above, buttermilk is a particularly preferred source of active XOR. Lyophilised buttermilk may be used. It may be preferable, however, for the buttermilk to be spray-dried by spraying the buttermilk into air at a temperature which is so selected that the activity of the XOR is wholly or at least substantially retained. In general, to avoid any detrimental effect on the activity of XOR, heat treatment steps that may be used in the treatment of XOR-containing compositions or formulations should preferably be such that the temperature of the composition or formulation does not exceed 65°C.

The composition may be in the form of a powder.

Advantageously, the composition further includes electron donors and/or electron acceptors. Examples of electron donors are purines and nitrogen-containing heterocyclic compounds, for example hypoxanthine, NADH. The nature of the electron donor may influence the rate of generation of bactericidal species. For example, use of hypoxanthine as electron donor results in a more rapid, but less prolonged generation of bactericidal species than NADH. Examples of electron acceptors are organic and inorganic nitrates and nitrites.

According to the invention, there is also provided, a method of making a formulation for use as feed, the

method comprising the step of adding a composition comprising active XOR.

The method advantageously comprises the steps of:

a. preparing a first portion of the formulation, the first portion comprising a composition including active XOR; and

b. preparing a second portion of the formulation, the first portion and second portion being separate from each other for subsequent mixing to form the formulation.

Advantageously, the first portion comprises buttermilk, preferably lyophilised buttermilk. As indicated above, the buttermilk may include additives.

Preferably, the second portion comprises a sterilised feed composition.

Also provided by the invention is the use of active XOR in the treatment of gastrointestinal (GI) infection. The invention also provides the use of active XOR in the treatment of Scours disease.

Additionally provided is the use of active XOR in the killing of bacteria.

The invention also provides a method of feeding a patient with enteral feed, in which the enteral feed includes active XOR and also a method of feeding a neonate with formula feed, in which the formula feed includes active XOR.

Also provided is a method of treatment of gastrointestinal infection using active XOR.

It will be appreciated that XOR used in accordance with the invention may be of any biological origin, for example of mammalian or other animal origin, or originating from a suitable micro-organism, for example,

aspergillus sp. XOR of ruminant origin offers the advantage of ready availability.

The invention will now be explained in more detail with reference to the accompanying drawings, of which:

Fig. 1 is a graph illustrating the effect of peroxynitrite on cell viability, under the conditions of Test 8(a);

Fig. 2 is a graph illustrating the effect of added peroxynitrite on cell colony growth in semi-skimmed-bovine milk, as determined in Test 8(b);

Fig. 3 is a graph illustrating the dependence of XO-mediated killing of cells as determined in Test 8(c);

Fig. 4 is a graph showing the effects of hypoxanthine addition on bacterial growth in pasteurised milk;

Fig. 5 is a graph showing the effect of XO concentration on bacterial growth rate;

Fig. 6 is a graph showing the dependence of cell growth inhibition on hypoxanthine concentration;

Fig. 7 is a graph showing the effect of oxypurinol on XO/hypoxanthine-related growth inhibition; and

Fig. 8 is a graph showing the effect of hypoxanthine at various concentrations on growth inhibition.

Determination of nitric oxide production

The production of nitric oxide in the following tests and examples was analysed using an ozone chemiluminescence assay in a continuous flow apparatus (Sievers NOA 280) that allows the real time analysis of NO production. The apparatus was modified to allow a constant stream of nitrogen to flow into the reaction chamber. Chemiluminescence data were collected by a data

acquisition system; the mean NO produced in parts per billion (ppb) was calculated from readings taken every second and shown as ppb or mV.

Progress curves, of molar production of NO against time, were calculated by taking into account the gas flow and the mean level of NO. Molar production of NO was expressed as ppb/sec or mV/sec. Reactions were carried out in a final volume of 1 ml at 37°C in an atmosphere of < 1% oxygen (Stathkelvin combination needle oxygen electrode, Diamond General Corp.).

The method used was as follows:

(a) Two clean 7ml bijous were obtained, one for each of the "substrates" and the "milk".

To the "substrates" bijou, 200µl of 100mM stock sodium nitrite was added together with 200µl of the 5mM reduced NADH to give an assay concentration of 20mM nitrite and 1mM reduced NADH.

To the "milk" bijou were added 600µl of milk sample to give an assay volume of 1ml.

(b) Using a flow rate of 200ml/min, each bijou mixture and a corresponding injection needle was degassed with nitrogen gas (N_2) for about 10 seconds and the bijou was capped.

(c) The reaction cell comprised a 7ml screw-cap bijou having three needle holes in its cap. A continuous flow of warmed N_2 (200ml/min flow rate) was injected into the bijou through one of the needle holes in the cap to give the required hypoxic conditions. The reaction cell was held at a temperature of 37°C in a water bath mounted on a magnetic stirrer. A magnetic flea was placed in the reaction cell to mix the samples once injected.

(d) Samples of NO were taken from the reaction cell by a needle that was connected to a Sievers Nitric Oxide

Analyser (NOA-280) and the results were recorded for analysis using a computer.

(e) At time (t) 0 minutes, the NOA-280 started the measurement of NO from the reaction cell. At t=1 minute, the contents of the "substrates" bijou were injected into the reaction cell using a 1ml syringe and the background NO was measured.

(f) At t=5 minutes, the contents of the "milk" bijou were injected into the reaction cell using a 1ml syringe and the release of NO was monitored for a further 20 minutes. The set up was such that the reaction cell was a sealed system having an inlet gas flow of 200ml/min and a sample extraction flow to the NOA-280 of 200ml/min. The steady-state generation of NO (which corresponded to a plateau region on the trace of the NO production) was noted to give the mV/s release of NO from the 1ml assay volume.

The samples were diluted with PBS where necessary to give an assay of 1ml for the test of NO generation.

Reagents used

The reagents used in the tests and examples described below were as follows:

1. Bovine xanthine oxidase (XOR) - Biozyme, Blaenavon, UK. This source of enzyme had a concentration of 10.7mg/ml and was batch 104AX
2. Sodium nitrite ($NaNO_2$) - Sigma Chemicals (Sigma-Aldrich Company Ltd.), Poole, UK. This was dissolved in 1X Phosphate Buffered Saline (PBS), pH 7.3, to the required concentration.
3. β-Nicotinamide Adenine Dinucleotide, reduced form, (β-NADH) - Sigma Chemicals.

4. Phosphate Buffered Saline (PBS) - Oxoid Ltd., Basingstoke, UK. Tablets were added in the proportion of 1 per 100ml of distilled water and mixed until thoroughly dissolved to give 1X PBS, pH7.3.

5. Oxypurinol - Sigma Chemicals. A stock 1mM solution was made up by adding 0.0015g to 0.25ml of 1M NaOH. This was mixed until the oxypurinol dissolved. Then 9.75ml of 1X PBS was added and the pH altered until pH7.3 was reached using drops of 1M Hl.

6. Diphenyliodonium (DPI) - ICN Biomedical. A stock 1mM solution was made up by adding 0.0032g to 10ml 1X PBS, pH7.3.

7. Formula milks:

a. PreAptimil with Milupan (2.4g protein/100ml) - Milupa, Trowbridge, UK.

b. Aptamil First with Milupan (1.5g protein/100ml) - Milupa.

c. Farleys First Milk - H.J. Heinz Co. Ltd., Hayes, UK.

d. Cow and Gate Premium (1.4g protein/100ml) - Cow and Gate, Trowbridge, UK.

e. Sma Gold 1.5g protein/100ml) - SMA Nutrition, Maidenhead, UK.

f. Sma Wysoy (1.8g protein/100ml) - SMA Nutrition.

8. Human Breast Milk. Samples obtained from subjects in the local area

9. Carton milk. Milk bought in pints from the local shop either as full milk or semi-skimmed milk (3.4g protein/100ml). Lordswood Dairy, Bristol, UK was the source of the milk.

10. Untreated cows' milk was obtained from a local farm

11. Buttermilk obtained from Waitrose (John Lewis Partnership, UK).

Test 1

The release of nitric oxide (NO) from a composition including pure bovine xanthine oxidase under hypoxic conditions was studied. The samples studied comprised reduced β-Nicotinamide adenine dinucleotide (NADH) and nitrite (NO_2^-) and pure bovine xanthine oxidase.

Bovine xanthine oxidase was diluted with PBS as indicated in Table 1 below to give 300µl of enzyme mixture. The enzyme mixture (300µl) was mixed with 700µl of a substrate mixture to give an assay volume of 1 ml. The substrate mixture comprised nitrite which was added to the assay to give a concentration of 1mM and NADH which was added to give a concentration of 0.3mM. Where, for example in Tests 2 to 4 below, additional components are added to the sample, the amount of PBS is adjusted accordingly to give an assay volume of 1 ml. The total XOR protein in the assay is shown in Table 1. The sample was placed in the reaction vessel of the NO determination apparatus under a nitrogen atmosphere and the generation of NO was measured and the results calculated as a steady state rate in mV/s. The rate of NO release for the different concentrations of XOR is also shown in Table 1.

Table 1

Volume XOR enzyme (µl)	Volume PBS (µl)	XOR protein in assay (µg)	NO release (mV/s)
0	300	0.0	15.10
2	298	21.4	50.05
5	295	53.5	76.35
10	290	107.0	128.55
15	285	160.5	187.20
20	280	214.0	225.75

Where the pure XOR is used in the samples, NADH was required as a substrate for the reduction of the nitrite to proceed. It is believed that in many cases, where the XOR is added to, for example a formula feed, the addition of a separate electron donor, for example NADH, will not be required because suitable substrate species will already be present in the feed and/or in the GI tract of the infant or adult to whom the feed is administered.

Test 2

Using the method described above in respect of Test 1, the effect of the variation in the concentration of NADH on the production of NO was investigated. 4mM NADH was diluted with PBS to give the relevant concentration. The results are shown in Table 2.

Table 2

XOR protein in assay (μg)	NADH concentration in assay (mM)	NO release (mV/s)
53.5	0.00	0.00
53.5	0.10	30.45
53.5	0.25	66.80
53.5	0.50	92.55
53.5	1.00	103.35
53.5	2.00	109.20
107.0	0.00	0.00
107.0	0.10	74.45
107.0	0.25	142.70
107.0	0.50	192.65
107.0	1.00	230.05
107.0	2.00	264.65

Test 3

Using the method described above in respect of Test 1, the effect of the variation in the concentration of nitrite on the production of NO was investigated. As for Test 1, the concentration of NADH was 0.3 mM. 1M nitrite (or 100mM nitrite, where appropriate for low concentrations) was diluted with PBS to give the relevant concentration. The results are shown in Table 3.

Table 3

XOR protein in assay (μg)	Nitrite concentration in assay (mM)	NO release (mV/s)
21.4	0	0.00
21.4	1	53.75
21.4	5	184.8
21.4	10	378.10
21.4	25	407.55
21.4	50	474.05

Test 4

The method of Test 1 was repeated with known inhibitors of XOR included in the assay to show that the NO generation was being catalysed by XOR. Two different inhibitors were used. Oxypurinol was used at a concentration of 100 μM. Oxypurinol is a molybdenum site-specific inhibitor. Diphenyliodonium (DPI) was used at a concentration of 100 μM. DPI is a FAD site inhibitor. The results are shown in Table 4.

Table 4

Inhibitor	NO release (mV/s)
100 μM Oxypurinol	0.00
100 μM DPI	0.00

There was no release of NO in the presence of the XOR inhibitors.

Test 5

Full fat milk was assayed with 20mM nitrite and 1mM NADH as described above in Test 1. The milk had a protein content of 3.4g/100ml. 600µl of milk was used in each test giving a protein content in each assay of 20.4mg.

The milk was taken and divided into two halves. One was kept at 4°C, and the other at -20°C. Samples of the milk were taken over several days and the generation of NO was investigated using the method described above. The results are given below in Table 5.

Table 5

Day	4°C - NO release (mV/s)	-20°C - NO release (mV/s)
1	77.57	73.00
2	75.50	90.05
3	68.67	52.65
6	626.1	73.35
8	Over 1000	39.20

It was found that the milk which was kept at 4°C started to go off after 3 days and that that was accompanied by a large increase in NO release which was not inhibited by 100µM oxypurinol or DPI. Therefore, when the milk goes off it is probable that bacteria in the milk are starting to produce NO from the nitrite. The milk kept frozen retained its activity and did not go off.

Samples of fresh full fat milk assay with 100µM Oxypurinol or DPI were tested and found to give no

detectable NO generation. Thus full fat milk does include a source of active XOR which produces NO under hypoxic conditions with nitrite and NADH.

Test 6

Human breast milk was assayed with 20mM nitrite and 1mM NADH using the method as described above. The human breast milk was collected on several days post partum and frozen at -20°C. 600µl of milk was used in each test. The generation of NO was investigated using the method described above. The results are given below in Table 6.

Table 6

Days post partum	XOR protein content (µg/ml)	NO release (mV/s)
7	411.26	40.45
30	683.05	60.75
36	561.49	42.48
66	305.52	27.80
158	110.84	29.80

Test 7

A selection of formula feeds (formula milks) were taken and assayed with 20mM nitrite and 1mM NADH as described above. The formula milks were tested for the generation of NO. The Cow and Gate Premium was left for 7 days after opening and then tested again. The results are shown in Table 7.

Table 7

Formula feed	NO release (mV/s)
Cow and Gate Premium (fresh)	0.00
Cow and Gate Premium (7 days old)	Over 1000
Milupa Aptimil First with Milupan	0.00
Sma Wysoy	0.00
Sma Gold	0.00
Farleys First	0.00
Milupa Preaptimil with Milupan	0.00

There was no activity found in any of the fresh formula milks. The Cow and Gate formula had gone off after 7 days and the NO release is probably related to bacteria (the activity was not inhibited by 100μM oxypurinol or DPI).

Test 8

To obtain cells used in this test, the infective bacterial strain Escherichia coli NCTC 86 (E.coli) was cultured on nutrient agar at 37° until colony formation occurred, usually overnight. This stock culture was used for subsequent experiments including sub-culturing in nutrient broth and plated onto agar weekly. Experimental cultures of E.coli were set up overnight in nutrient broth from single colonies on an agar plate. Cells were harvested and counted using a standard curve of known absorbance at 470nm against viable cell count. Peroxynitrite was generated by the method of Crow et al, Biochem. 34, p.3544-3552 (1995). In accordance with that

method, a mixture of sodium nitrite and hydrogen peroxide was reacted under acid conditions then immediately quenched by the addition of sodium hydroxide. The concentration of $ONOO^-$ formed was measured using an absorption coefficient of 1670 M^{-1} cm^{-1} in a spectrophotometer at a wavelength of 303nm. Solutions of different concentrations for use in (a) below were obtained by dilution of the product solution using PBS.

8(a) Peroxynitrite effect on cell viability

Cells cultured as described above were diluted to suitable working concentrations (10^4 - 10^5 Cells Ml^{-1}) in sterile phosphate buffered saline (PBS). $ONOO^-$ at a range of concentrations from 100μm to 0.01μm was added as a bolus dose to cells and incubated at room temperature for ten minutes. Aliquots were taken from the cell cultures and plated on to nutrient agar and incubated in a warm room at 37°C overnight. Viable cells formed colonies on the agar and were counted. The number of colonies formed was related to an untreated control and a graph of the results is shown in Figure 1, which illustrates the effect of $ONOO^-$ on cell viability. The decrease in viable count with increasing concentration of $ONOO^-$ in Fig. 1 is indicative of a profound inhibitory effect.

Peroxynitrite has also been shown to reduce viability in S. enteritidis under similar conditions to those indicated above. IC_{50} values of peroxynitrite in respect of E. coli and S. enteritidis (that is, the concentration that reduces viability to 50% of control viable count of the respective cell type) of 1.402 and 2.026μM were determined under the conditions used.

Peroxynitrite-medicated killing has also been indicated in the case of Gram positive bacteria, in a test in which

incubation of Staphylococcus aureus with SIN-1 (3-morpholinosydnonimine, which releases both superoxide and NO simultaneously on hydration) showed reduced growth in dependence on the amount of added SIN-1.

8(b) Effect of peroxynitrite addition to bovine milk

Commercially produced bovine milk was purchased in semi-skimmed form. Aliquots were taken on day one of experimentation and plated onto nutrient agar and grown overnight at 37°C. The number of colonies formed following incubation was counted. The remaining milk was divided into three portions after removal of those aliquots. No peroxynitrite was added to the first portion, which was the control. A single bolus dose of 100μM $ONOO^-$ was added to the second portion on day one, and the third portion was treated by addition of a daily bolus dose of 100μM $ONOO^-$. The portions were stored during the four days of the test at a temperature of 4°C. Each day, aliquots were taken from each portion and cultured overnight at 37°C for viable count determination.

The results are shown in Fig. 2 and demonstrate that $ONOO^-$ addition to milk caused a reduction in the number of colony forming units (CFU) over time compared to the control. The single bolus addition reduced the CFU significantly ($p<=0.01$) and the multiple dose $ONOO^-$ reduced the CFU significantly below control ($p<=0.01$) and also significantly below the single dose $ONOO^-$ ($p<=0.05$). Although the contaminants were not formally identified, it was postulated that they included at least Lactobacilli sp., that being a cell type often present in milk.

8(c) Effect of xanthine oxidase derived species on cell viability

E.coli were cultured as described above. Aliquots were taken and incubated with a reaction system consisting of bovine Xanthine oxidase (XO) (53.2 μgml^{-1}), nicotinamide adenine dinucleotide in reduced form (NADH) (300μM), sodium nitrite, ($NaNO_2$) 1mM and oxygen at a range of concentrations. Desired oxygen concentrations were generated by delivery into the system of a mixture of oxygen and nitrogen in appropriate proportions and determined using a Clark-type O_2 electrode. This reaction was followed at 37°C for 30 min with mixing before an aliquot was taken and plated onto agar and incubated in a warm room at 37°C. Viable cells formed colonies on the agar and were counted. Viable cell counts were performed in triplicate and the results expressed as a percentage viable count in each case related to a non enzyme control of the same oxygen concentration. The results are shown in Fig. 3, which suggest that XO-mediated killing of cells is occurring. The most effective killing (indicated by the lowest viable cell count) is at an oxygen concentration of approximately 3% of saturation. The range of oxygen tensions used covers those in which XO has previously been considered to be active, namely superoxide generation (21% O_2 saturation) and nitric oxide production (0% O_2 saturation). A certain amount of killing is seen at both of these extremes as compared with control samples. However, it is only at an intermediate oxygen concentration that the greatest amount of killing is observed. This suggests a role for peroxynitrite mediated killing which has been generated in this system by the enzyme xanthine oxidase.

Corresponding data obtained in respect of S. enteritidis indicated a peak killing oxygen concentration of 0% for that cell type. For both E. coli and S. enteritidis the viability increases with oxygen concentration with little or no killing above about 8% oxygen, although some limited killing (about 5% and about 10% for the respective cell types) is again observed at higher oxygen concentrations of about 21%.

Replacement of NADH in the above method by 100μM hypoxanthine, with a sodium nitrite concentration of 2.5μM led to slightly higher peak killing oxygen concentrations (6.4% for E. coli and 1.5% for S. enteritidis). More limited killing was also observed using xanthine instead of hypoxanthine. The XO/hypoxanthine combination was also found to reduce the growth rate of Lactobacillus in a dose dependent manner for both hypoxanthine and XO. Addition to the XO/hypoxanthine system of superoxide dismutase at oxygen concentrations in the range of from 0 to 2% was found to increase the amount of measurable NO (as a result of removal of superoxide by superoxide dismutase), providing a further indication of the hypothesis that peroxynitrite formation from NO and superoxide occurs in the XO/hypoxanthine system.

8(d) Effect of peroxynitrite scavenger on bacterial growth

E. coli were seeded into nutrient broth at 2.10^7 cell ml^{-1} and incubated at 37°C under atmospheric air conditions (that is, 21% oxygen saturation). Four separate test samples of volume 1 ml were prepared by addition at 1 hour of incubation as follows:

1 Peroxynitrite at concentration 100μM.

2 Peroxynitrite at 100μM and quercetin (peroxynitrite scavenger) at 100μM.

3 Xanthine oxidase (53.2μg) and xanthine at 100μM.

4 Xanthine oxidase (53.2μg) and NADH at 100μM.

Corresponding controls were also prepared, and the growth curves generated over time determined by monitoring absorbance. Sample (1) showed strong killing, but the presence of quercetin (sample (2)) had a clear effect in reducing killing, pointing towards peroxynitrite mediation of killing. XO/NADH (sample (4)) and XO/xanthine (sample (3)) showed limited, but significant, growth retardation effects.

The results of parts (a), (b) and (d) of this test appear to confirm the bactericidal potency of the peroxynitrite species. Part (c) above supports the hypothesis that, under appropriate conditions, XOR can catalyse production both of superoxide and of NO, interaction of those two products giving rise to peroxynitrite, and possibly other interaction products that may have similar bactericidal activity to peroxynitrite. The oxygen concentrations of under 8% at which maximum killing was observed is believed to be similar to that in the neonatal gut.

8(e) Effect of hypoxanthine addition on bacterial growth in pateurised milk.

Pasteurised semi skimmed milk 1 ml was aliquoted into 7 sterile plastic bijou bottles comprising control and treated hypoxanthine (Hxan) groups. To the control group at day 0 was added 10μl of sterile ddH_20 and to the treated group 10μl of hypoxanthine solution was added to give a final concentration of 100μM. The samples were stored refrigerated at 4-8°C until required on a specific

day when a control and treated sample was incubated at 37°C for 30 minutes. A 100µl aliquot was taken from each sample and spread onto nutrient agar which was then incubated over night at 37°C. The total number of colonies formed regardless of type was counted and expressed for each treatment as the colony forming units per ml of the original test sample (CFU ml^{-1}). The results are shown in Fig. 4.

The control group showed significant colony formation from day 1. The effect of the addition of hypoxanthine was to reduce the total number of colonies formed for the length of the experiment (8 days).

8(f) Effect of XO and hypoxanthine incubation on bacterial growth rate.

Bacteria were seeded into nutrient broth (NB) and grown over night at 37°C. The culture was then counted and normalised to give a final cell concentration of $1.8x10^7$ cells $well^{-1}$ into the wells of a 96 well plate in fresh nutrient broth. The optical density of each well was monitored every 15 minutes as a measure of the growth rate of each bacterial species. Growth curves were generated for each species and the maximal rate of growth (logarithmic phase) was measured. Cells were treated to a range of experimental conditions in which the growth rate of cells was related to a control of untreated cells. The results were expressed as a percentage of the growth rate of the control cells.

(i) At a fixed concentration of hypoxanthine (100µM) a range of purified XO protein concentrations were added at time 0 minutes. The optical density was followed and the growth rate calculated. The effect of enzyme concentration is shown in Fig. 5

for *Staphylococcus aureus* 6751 and *Lactobacillus casei* 6375.

The growth rate of both Staphylococcus and Lactobacilli was reduced compared to the control. Lactobacillus growth rate was reduced with a half maximal concentration of XO (that is, the concentration at which the cell growth was 50% of control) being about 14µg. A less marked growth inhibition was observed for Staphylococcus.

(ii) The growth rate was also measured in relation to the concentration of hypoxanthine, using 30µg of XO with cells seeded as described above. Fig. 6 shows the effect of hypoxanthine concentration on bacterial growth. Hypoxanthine in the presence of XO reduced the growth rate of both bacterial species with greatest effect on the Lactobacillus. The half maximal hypoxanthine dose at 30µg XO was 103.5µM.

(iii) To show that the effect of XO and Hypoxanthine addition was due to the enzymically derived products, oxypurinol at a range of concentrations was added to the cells in the presence of 30µg XO and 200µM hypoxanthine. Fig. 7 shows its effect on the growth rate of Lactobacillus.

Oxypurinol had no effect on growth rate when added at the highest concentration when added alone. However the effect of oxypurinol was to reduce the effect of XO/hypoxanthine growth inhibition in a dose dependent manner.

(iv) The effect of XO hypoxanthine addition was measured on the growth rate of a range of bacterial species with 30µg XO at varying

hypoxanthine concentrations using cells seeded as described above. The results of this study are shown in Fig. 8.

8(g) Effect of H_2O_2 and peroxynitrite on the growth rate of bacteria

To determine the effect of possible enzymically generated radical species cells were grown in the presence of hydrogen peroxide or peroxynitrite. Cells of various species were grown as previously described and a bolus addition of either H_2O_2 or peroxynitrite was added at the beginning of logarithmic growth, previous experiments having showed this treatment as the most effective. The cell growth was determined and plotted against the concentration of H_2O_2 or peroxynitrite, as appropriate, and the half maximal concentration of H_2O_2 or peroxynitrite was determined. The results are summarised in Table 8, in which corresponding half maximal concentrations for the XO/hypoxanthine system are also given.

Table 8

	Half maximal concentration		
Species	XO/Hxan (μM Hypoxanthine)	H_2O_2 (μM)	ONOO - (μM)
Bacillus sp	39.8	77.6	
Micrococcus sp	25.1	19.5	
Lactobacillus casei	103.5	304.8	55
Staphylococcus aureus		–	290
E. coli			1.4
Salmonella enteritidis			2.0

As shown in Table 8, the half maximal concentrations for peroxynitrite in respect of Lactobacillus casei is much lower than for H_2O_2. In respect of S. aureus, the growth inhibition in the presence of H_2O_2 was very limited, with 50% growth reduction not being observed at the concentration ranges used.

The following Example illustrates the invention:

Example 1

The effect of the addition of XOR to formula feed on the generation of NO was investigated. Different compositions comprising one or more of buttermilk, formula feed, XOR, nitrite and NADH were studied under hypoxic conditions.

The samples were tested for NO generation using the method described above.

To the "milk" bijou were added either 598µl Cow and Gate formula feed and 2µl XOR **or** 100µl buttermilk and 500µl 1X PBS to give a total volume in the "milk" bijou of 600µl in each case.

Table 9

Sample	20mM nitrite added?	1mM NADH added?	NO release (mV/s)
Formula feed and XOR	Yes	Yes	247.3
Formula feed and XOR	No	No	0.0
Formula feed and XOR	Yes	No	0.0
Formula feed and XOR	No	Yes	0.0
Formula feed and 1X PBS	Yes	Yes	0.0
Buttermilk	Yes	Yes	379.1

Table 9 shows that where no XOR is added to the formula feed, there is no generation of NO. That is consistent with the applicant's findings that formula feeds do not contain active XOR.

The addition of XOR to the formula feed in the presence of nitrite and NADH led to the generation of NO. The omission of either the nitrite or the NADH gave no NO generation. As indicated above, it is believed that both a source of nitrite and an electron donor substrate will be available *in vivo* in the GI tract and that in many cases specific further addition of nitrite and/or NADH (or other substrate) will not be required.

Buttermilk alone, without the addition of any of XOR, nitrite or NADH, led to the generation of NO. As indicated above, buttermilk contains active XOR as well as a source of nitrite, and an electron donor substrate.

Claims

Claims:

1. A formulation for use as a bactericidal agent in the human or animal digestive system, the formulation including active xanthine oxidoreductase (XOR).

2. A formula feed formulation or enteral feed formulation for administration to a human or animal, the formulation including active xanthine oxidoreductase (XOR).

3. A formulation according to claim 2, in which the formulation is for use as formula feed.

4. A formulation according to any one of claims 1 to 3, in which the concentration of XOR exceeds the normal physiological concentration of XOR.

5. A formulation according to any one of claims 1 to 4, in which the formulation includes from 50 to 150μg/ml of XOR.

6. A formulation according to any one of claims 1 to 5, in which the formulation includes buttermilk, the buttermilk including active XOR.

7. A formulation according to any one of claims 1 to 6, in which the formulation is in liquid form.

8. A formulation according to any one of claims 1 to 7, the formulation further including one or more electron donors.

9. A formulation according to any one of claims 1 to 8, the formulation further including one or more electron acceptors.

10. A combination product for use in the preparation of a formulation according to any one of claims 1 to 9, in which the product comprises two separate portions, the first portion including active XOR and the second portion comprising substantially no active XOR.

11. A combination product according to claim 10, in which the second portion is in the form of a powder.

12. A formulation according to claim 10 or claim 11, in which the second portion has been heat treated.

13. A formulation according to any one of claims 10 to 12, in which the first portion has been pasteurised.

14. A formulation according to any one of claims 10 to 13, in which the first portion is in a first container, the second portion is in a second container.

15. A composition for addition to a formulation for use as feed, the composition comprising active XOR in combination with one or more electron donors and/or one or more electron acceptors.

16. A composition according to claim 15, the composition comprising buttermilk.

17. A composition according to claim 15 or claim 16, the composition being in the form of a powder.

18. A method of making a formulation for use as feed, the method comprising the step of adding a composition comprising active XOR.

19. A method according to claim 18, the composition being according to any one of claims 15 to 17.

20. A method according to claim 19 or claim 20, the method comprising the steps of:

a. preparing a first portion of the formulation, the first portion comprising a composition including active XOR; and

b. preparing a second portion of the formulation, the first portion and second portion being separate from each other for subsequent mixing to form the formulation.

21. A method according to claim 20, in which the first portion comprises lyophilised buttermilk.

22. A method according to claim 20 or claim 21, in which the second portion comprises a treated feed composition.

23. Use of active XOR in the treatment of gastrointestinal (GI) infection.

24. Use of active XOR in the treatment of Scours disease.

25. Use of active XOR in the killing of bacteria.

26. Method of feeding a patient with enteral feed, in which the enteral feed includes active XOR.

27. Method of feeding an infant with formula feed, in which the formula feed includes active XOR.

28. A formulation comprising XOR substantially as described in Example 1 herein.

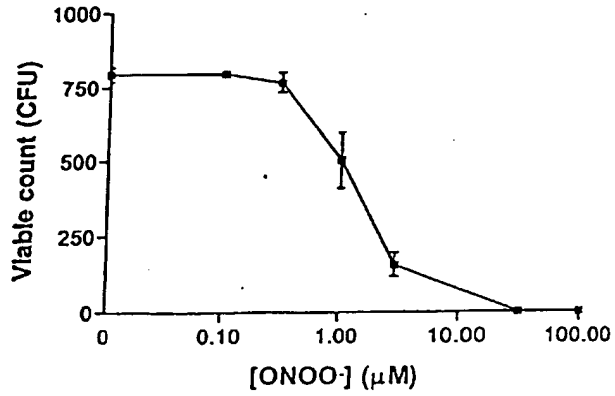
1000
750
500
250
0
Viable count (CFU)
0
0.10
1.00
10.00
100.00
[ONOO-] (μM)

Fig. 1

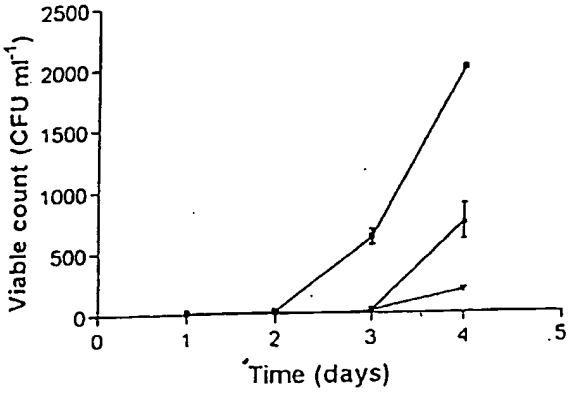
2500
2000
1500
1000
500
0
Viable count (CFU ml-1)
0
1
2
3
4
5
Time (days)
Control
ONOO- x1
ONOO- x6

Fig. 2

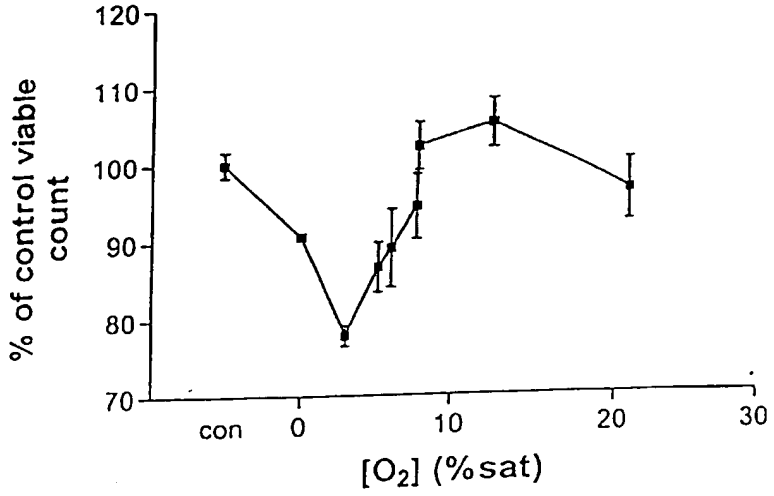
120
110
100
90
80
70
% of control viable count
con
0
10
20
30
[O2] (%sat)

Fig.3

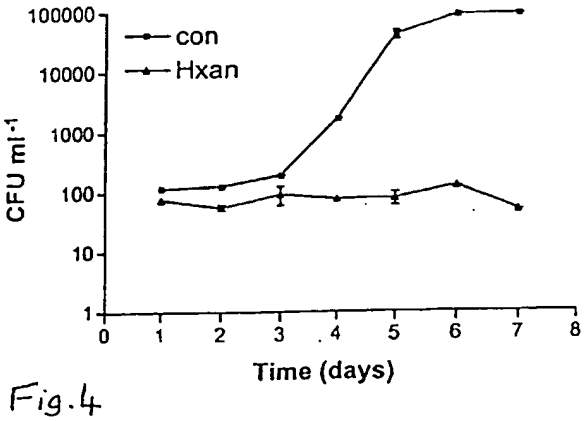
100000
10000
1000
100
10
1
CFU ml-1
con
Hxan
0
1
2
3
4
5
6
7
8
Time (days)

Fig.4

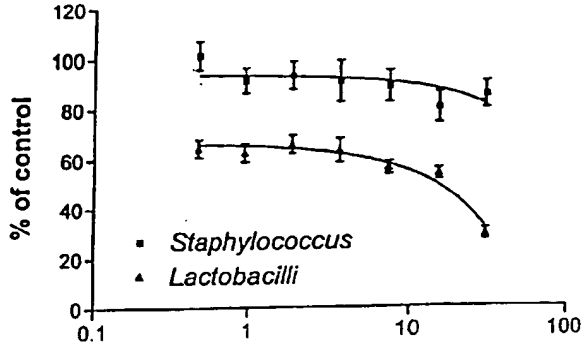
120
100
80
60
40
20
0
% of control
Staphylococcus
Lactobacilli
0.1
1
10
100
[XO] (µg)

Fig.5

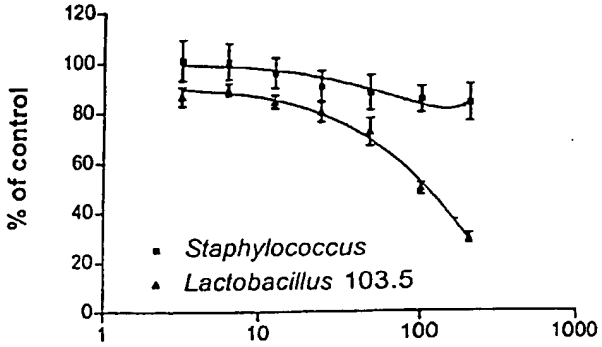
120
100
80
60
40
20
0
% of control
Staphylococcus
Lactobacillus 103.5
1
10
100
1000
[Hypoxanthine] (µM)

Fig.6

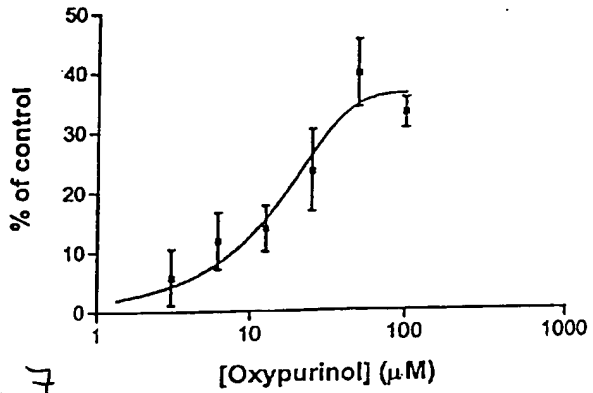
% of control
50
40
30
20
10
0
1
10
100
1000
[Oxypurinol] (μM)

Fig. 7

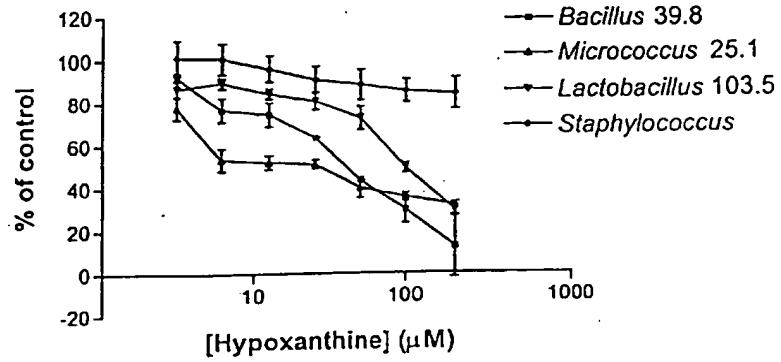
% of control
120
100
80
60
40
20
0
-20
10
100
1000
[Hypoxanthine] (μM)
Bacillus 39.8
Micrococcus 25.1
Lactobacillus 103.5
Staphylococcus

Fig. 8